

COMBINATION OF NO SYNTHASE INHIBITOR(S)
AND METABOLIC ANTIOXIDANT(S)

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5 The invention relates to a pharmaceutical composition containing, as active ingredient, one or many substance(s) which interfere(s) with the synthesis of nitrogen monoxide by inhibition of NO synthase and one or many metabolic antioxidant(s) which intervene(s) in the redox status of thiol groups, and optionally a pharmaceutically acceptable support. The invention also relates to a product containing one or many NO synthase inhibitory substance(s) and one or many metabolic antioxidant(s) which intervene(s) in the redox status of thiol groups, as a combination product, in separated form, of these active ingredients.

10 A pharmaceutical composition and a product according to the invention are useful in the treatment of pathologies where nitrogen monoxide and the metabolism of antioxidants (such as vitamin E or glutathione) as well as the redox status of the thiol groups are involved, and in particular :

- 15 . cardiovascular and cerebrovascular disorders comprising, for example, migraine, arterial hypertension, cardiac or cerebral infarctions of ischemic or haemorrhagic origin, ischemias and thromboses ;
- . septic shock, radioactive irradiation, solar radiation, organ transplants ;
- . disorders of the central or peripheral nervous system such as, for example, neurodegenerative diseases where cerebral infarctions, senile dementia, including Alzheimer's disease, Huntington's chorea, Parkinson's disease, Creutzfeld-Jacob's disease, prion diseases, amyotrophic lateral sclerosis, but also pain, cerebral or bone marrow traumas, addiction to opiates, alcohol and addictive substances, erective and reproductive disorders, cognitive disorders, encephalopathies, depression, anxiety, schizophrenia, epilepsy, sleeping disorders, eating disorders (anorexia, bulimia, etc.) can be mentioned in particular ;
- 20 . proliferative and inflammatory diseases such as, for example, cancer, atherosclerosis, pulmonary hypertension, glomerulonephritis, portal hypertension, cataracts, psoriasis, arthrosis and rheumatoid arthritis, fibroses, amyloidoses, inflammations of the gastrointestinal system (colitis, Crohn's disease) or of the pulmonary system and airways (asthma, sinusitis) as well as contact or delayed hypersensitivities ;
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5 In a pharmaceutical composition or product according to the invention, the NO synthase inhibitor and the metabolic antioxidant can be present in doses which can be identical or different. The doses are chosen according to the compounds combined with appropriate diluents or excipients.

Among NO synthase inhibitors, compounds of amino-acid type, non amino-acid type and aromatic amine type can be defined. NO synthase inhibitors of amino-acid type can be compounds as described in the Applications WO 95/00505, WO 94/12163, WO 96/06076, WO 98/28257, or L-arginine, ornithine, or lysine derivatives as described in the Applications WO 93/24126, WO 95/01972, WO 95/24382, WO 95/09619 and WO 95/22968 (the amino acids are excluded from this class as they have no activity in the NO system ; L-arginine alone has an activity : this is the natural substrat of NO synthase).

25 NO synthase guanidine inhibitors can be compounds as defined in the Applications
WO 95/28377, WO 91/04023, WO 94/21621, WO 96/18607 and WO 96/18608.

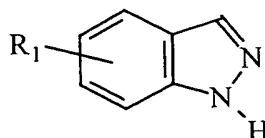
NO synthase isothiourea inhibitors can be compounds as defined in the Applications WO 95/09619, WO 96/09286, WO 94/12165, WO 96/14842, WO 96/18607, WO 96/18608, WO 96/09286, EP 717040 and EP 718294.

30 NO synthase nitro- or cyano-aryl inhibitors can be compounds as defined in the Application WO 94/12163.

NO synthase amino-pyridine or amino-pyrimidine inhibitors can be compounds as defined in the Applications WO 94/14780, WO 96/18616, WO 96/18617, WO 98/45294, WO 98/24766, WO 00/02860, JP 98/001470, JP 98/120654 and JP 98/036351.

- 5 NO synthase amidine inhibitors can be compounds as defined in the Applications WO 95/11014, WO 96/01817, WO 95/05363, WO 95/11231, WO 96/14844, WO 96/19440, WO 98/42696, WO 98/58934, WO 98/50380, WO 98/50382, JP 98/265450, or compounds such as N-phenyl-2-thiophenecarboximidamide.

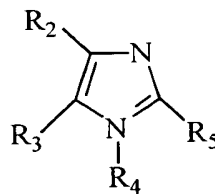
- 10 NO synthase indazole inhibitors can be compounds as defined in the Application WO 98/02442 or compounds of general formula I_A



I_A

in which R₁ represents one or more substituents chosen from a hydrogen atom, the nitro, halo, lower alkyl or lower alkoxy radical.

- 15 NO synthase imidazole inhibitors can be compounds of the general formula II_A



II_A

- 20 in which R₂ and R₃ represent, independently, a hydrogen atom, halo, hydroxy, amino, alkyl or alkoxy radical, or R₂ and R₃ are linked together and form the phenyl radical condensed with the imidazole ring, the phenyl radical being optionally substituted by one or more substituents chosen from hydroxy, trifluoromethyl, halo, carboxy, lower alkyl, lower alkoxy or lower alkenyl radicals ; R₄ represents a hydrogen atom, a lower alkyl, amino, lower alkyl amino or phenyl radical, the phenyl radical being optionally substituted by one or more substituents chosen from
- 25 hydroxy, trifluoromethyl, halo, carboxy, lower alkyl, lower alkoxy or lower alkenyl radicals ; R₅ represents the hydrogen atom, a lower alkyl, amino, lower alkyl amino radical.

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More preferably, a subject of the invention is also a composition or a product as
5 defined above, characterised in that the NO synthase inhibitor is an inhibitor of the
neuronal and/or inducible NO synthase.

15 All the technical and scientific terms used in the present text have the meanings known to a person skilled in the art. Moreover, all patents (or patent applications) as well as other bibliographical references are incorporated by way of reference.

EXPERIMENTAL PART :

20 The activity of the compounds of the invention was evaluated *in vivo* on a model of neurotoxicity with MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine). The administration of MPTP produces a syndrome similar to Parkinson's disease resulting in a degeneration of the dopaminergic nigrostriatal neurons. This was observed in man, primates and mice [Langston JW and Ballard PA, Parkinson's disease in a chemist working with 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine, N.Engl.J.Med. 309, 310 (1983) ; Burns RS et al., A primate model of parkinsonism : selective destruction of dopaminergic neurons in the pars compacta of the substantia nigra by N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, Proc. Natl. Acad. Sci. U.S.A. 80, 4546-4550 (1983), Heikkila, RE. et al., Dopaminergic neurotoxicity of 1-methyl-4-phenyl-1,2,5,6- tetrahydropyridine in mice, Science, 224, 1451-1453 (1984)].

Mice (C57BL6) weighing 15-25 g are injected three times with 15-20 mg/kg of MPTP by intraperitoneal route at 2-hour intervals. The products are injected by oral

route 90 minutes before each injection of MPTP and 90 min after the last and 24 hours after the first injection of MPTP. The mice are sacrificed 24 hours after the last injection of MPTP. The striatum is removed and its dopamine level is measured by high-performance liquid chromatography coupled with electrochemical detection.

- 5 The effectiveness coefficient of the compounds is calculated according to the ratio : dopamine level of the product group + MPTP / dopamine level of the MPTP group only. A product for which the effectiveness coefficient is \geq to 1.5 is considered beneficial.

Let A be the NO synthase inhibitor and B the metabolic antioxidant.

10 Example 1

Compound AB, combination of the active ingredients A and B. Compound A : N-phenyl-2-thiophenecarboximidamine, powerful NO synthase inhibitor. Compound B : reduced form of lipoic acid, metabolic antioxidant.

Compound of Example 1 : 4 groups of animals are constituted as follows :

- 15 Group 1 : treated with MPTP.
Group 2 : treated with A (3 mg/kg) + MPTP.
Group 3 : treated with B (10 mg/kg) + MPTP.
Group 4 : treated with AB + MPTP.

Group No.	Dopamine level ng/mg of tissue	Effectiveness coefficient
1	3.24	-
2	3.77	1.16
3	3.81	1.17
4	5.21	1.60

- 20 The results show that the lipoic acid, in reduced form, used as metabolic antioxidant at the dose of 10 mg/kg is ineffective for protecting the animal against the fall in dopamine which occurs after injection of MPTP. The N-phenyl-2-thiophenecarboximidamine used as NO synthase inhibitor at the dose of 3 mg/kg is

also ineffective. In contrast, the combination of the two compounds proves effective in restoring the dopamine level of the animals subjected to the MPTP neurotoxicity.

Example 2

- Compound AB, combination of the active ingredients A and B. Compound A :
5 N^Gnitro-arginine, powerful inhibitor of constitutive and inducible NO synthases.
Compound B : reduced form of lipoic acid, metabolic antioxidant.

Compound of Example 2 : 4 groups of animals are constituted as follows :

- 10 Group 1 : treated with MPTP.
Group 2 : treated with A (3 mg/kg) + MPTP.
Group 3 : treated with B (10 mg/kg) + MPTP.
Group 4 : treated with AB + MPTP.

Group No.	Dopamine level ng/mg of tissue	Effectiveness coefficient
1	4.11	-
2	6.98	1.69
3	4.48	1.09
4	8.65	2.1

The N^Gnitro-arginine used as an inhibitor of NO synthases, effective at the dose of 3 mg/kg, has an increased effectiveness when it is combined with lipoic acid.

- 15 The experimental results of Examples 1 and 2 therefore show a potentializing effect,
even a synergy between the two types of compounds.